Reports and Letters

Improved Method for Isolation of Adenosine Di- and Triphosphates

The technique used by Albaum (*1*) for isolation of adenosine polyphosphates from higher plants has been applied in the present studies to conidia of *Neurospora sitophila* (Mont.) Shear and Dodge. In order to extract acid-soluble phosphates quantitatively from conidia, however, it was necessary to repeat the extraction five or six times with three volumes of 5-percent trichloroacetic acid (TCA). This resulted in a large volume of liquid and very dilute solutions of phosphates. Under these conditions, 50 percent or more of the orthophosphate and adenosine diphosphate present in the original TCA extracts was lost in the barium isolation procedure. No efficient means have been found for complete breakage of the conidia; therefore, the volume of TCA could not be diminished by homogenation, which is possible when many other source materials are used.

A search was made, therefore, for a cation that would precipitate phosphates more nearly completely than barium in dilute solutions and that would still lend itself to convenient isolation and purification of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) by adsorption on anionic exchange resin by the method of Cohn and Carter (*2*). Of the several cations tested, Zn^{++} was found to be most suitable. With zinc, the step involving dissolution of the original precipitate in HCl could be eliminated because the zinc phosphates are soluble in 1*N* NH_4OH, and zinc, in this solution, passes readily through the resin column, whereas orthophosphate, ATP and ADP ions are adsorbed.

For the purpose of comparing zinc and barium as precipitating agents, isolation of phosphates from one-half of TCA extracts, usually about 200 ml, was done with barium as described by Albaum (*1*), and from the other half with zinc as follows. Five milliliters of 25-percent zinc acetate solution were added to the extract. The solution was cooled to 0°C and the precipitate was collected by centrifugation. The precipitate was washed once with 15 ml of cold distilled water to remove excess TCA and then resedimented. It was dissolved in 15 ml of 1*N* NH_4OH and passed several times through a 2- to 3-cm column of Dowex No. 1 anionic exchange resin in a 100-ml burette. This was followed by two 15-ml portions of 1*N* NH_4OH to wash out the zinc. NH_4OH was removed by several 15-ml portions of distilled water. Adenosine monophosphate (AMP), ADP, and ATP were eluted differentially according to the method of Cohn and Carter (*2*) and measured spectrophotometrically at 260 mμ using adenylic acid as the standard. Most of the inorganic orthophosphate was eluted along with ADP and was measured by the colorimetric method of Lowry and Lopez (*3*), with which inorganic phosphate can be determined in the presence of labile phosphate esters.

Data are given in Table 1 from two experiments in which the yields of orthophosphate, AMP, ADP, and ATP were compared when the two procedures were used. The zinc precipitation method yielded two to nine times as much of the various phosphate fractions in the final analyses as did the barium method. Precipitation of the phosphates with zinc revealed that ADP and ATP were present in conidia in essentially equal quantities, whereas precipitation with barium would lead one to the conclusion that ADP was present in much smaller quantities than ATP. The ratio of ADP to ATP in conidia was thus considerably higher than that found in other tissues where barium precipitation was used (*1, 4*). It is possible, since prolonged extraction with TCA was necessary, that some ATP was hydrolyzed to ADP. However, the yield of ATP was also always greater with zinc than it was with barium; hydrolysis of zinc-ATP would not account for the observed differences in ADP.

Table 1. Comparative yields of inorganic phosphate, AMP, ADP, and ATP from conidia of *Neurospora sitophila* with barium and zinc as precipitating agents. In experiment No. 1, 4.78 g (dry weight) of spores were extracted; in experiment No. 2, 9.56 g (dry weight) of spores were extracted.

Compound	Expt. 1 Barium (μ*M*)	Expt. 1 Zinc (μ*M*)	Expt. 2 Barium (μ*M*)	Expt. 2 Zinc (μ*M*)
Inorganic phosphate*	20.1	108.6	169.5	407.6
AMP	Trace	Trace	Trace	Trace
ADP	0.5	3.0	1.9	17.5
ATP	2.6	3.9	6.8	15.9

* Calculated as phosphoric acid.

The identities of ADP and ATP were confirmed by paper chromatograms developed according to the method of Cohn and Carter (*2*). Both compounds exhibited the same chromatographic properties as yeast ATP and ADP.

It would appear that use of zinc in place of barium might give a better quantitative value for ATP and ADP in tissues and cells other than fungus spores, especially when the quantity of cells is small and when large volumes of TCA must be used for quantitative extraction of the phosphates. A complete report on the application of the modified method as described here to studies on phosphorus metabolism in germinating fungus spores will be submitted for publication elsewhere.

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References

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Radiocarbon Concentration in Modern Wood

Redeterminations of the absolute C^{14} concentration in wood carbon and of its variations since the industrial revolution became widespread in the late 19th century were carried out (*1*) by means of proportional counting of acetylene as described previously (*2*). No direct determination of the counting efficiency was made, but the counting rates were compared with those from material containing a mass-spectrometrically determined amount of artificial C^{14}, obtained from the National Bureau of Standards through the courtesy of H. H. Seliger.

Indications of a decrease in the specific C^{14} activity of wood at time of growth during the past 50 yr had been found previously (*3*). The decrease amounted to about 3.4 percent in two trees from the east coast of the United States. A third tree, from Alaska, investigated at that time, showed a smaller effect. The decrease can be attributed to the introduction of a certain amount of C^{14}-free CO_2 into the atmosphere by artificial coal and oil combustion and to the rate of isotopic exchange between atmospheric